Analysis and antioxidant capacity of the phenolic compounds from argan fruit (Argania spinosa (L.) Skeels)

Analysis and antioxidant capacity of the phenolic compounds from argan fruit (*Argania spinosa* (L.) Skeels)

Hanae El Monfalouti[1,2], Zoubida Charrouf[1], Simona Belviso[3], Daniela Ghirardello[3], Bernardo Scursatone[3], Dominique Guillaume[2], Clément Denhez[2] and Giuseppe Zeppa[3]

[1] Laboratoire de Chimie des Plantes, Faculté des Sciences, Université Mohammed V-Agdal, Rabat, Morocco

[2] Laboratoire de Chimie Thérapeutique, Université de Reims Champagne Ardenne, Reims, France

[3] Department of Valorization and Exploitation of Agroforestry Resources, Food Microbiology and Technology Sector, University of Turin, Via Leonardo da Vinci, Grugliasco (TO), Italy

Abstract

Polyphenol composition of the shell, pulp, and roasted and unroasted kernels of the argan fruit was qualitatively and quantitatively determined by HPLC coupled with electrospray negative ionization ion trap MS (HPLC-ESI-MS). Eleven phenolic compounds were identified. Unroasted kernels and shell contained various polyphenols but the pulp was characterized by a very high amount of total polyphenols (75.78 mg of gallic acid equivalent/g). The radical scavenging (DPPH· method) and antioxidant activity (ABTS method) of each fruit part was also determined. Argan fruit pulp showed the highest radical scavenging activity (0.17 0.005 *m*M Trolox equivalent (TE)/mg) and antioxidant activity (0.375 0.07 *m*M TE/mg). Therefore, argan fruit polyphenols are good candidates to be exploited as health supplements and nutraceuticals.

Keywords: Antioxidant / Argan oil byproduct / Argan tree fruit / HPLC-ESI-MS / Polyphenol

1 Introduction

The argan tree (*Argania spinosa* (L.) Skeels) of the Sapotaceae family exclusively grows in south-western Morocco where it covers an area of about 320 000 square miles and plays an essential function for the sustainable development of this part of the world [1]. Fresh argan fruit is composed of latex-rich sticky pulp that covers a big stone where two or three kernels are located. When the fruit is dry, it can be peeled by scratching and its black pulp can be collected. Kernels of argan fruit are used for the preparation of argan oil, the basic ingredient of the amazigh diet [2]. Traditionally, argan oil is prepared by argan forest dwellers who manually collect then pulp argan fruit to get argan nuts whose shell is subsequently broken. Resulting argan kernels are amassed, then eventually roasted, then mechanically cold-pressed to afford virgin argan oil of beauty (unroasted kernels) or edible (roasted kernels) grade. Each oil type possesses its own set of pharmacological properties [3, 4]. Since 2010, annual argan oil production has exceeded 5000 tons. By-products generated during the oil preparative process are the nut-shell, the fruit pulp, and the press-cake. Two types of press-cake exist because kernels can be roasted or not. For the moment, fruit pulp and press-cakes that are palatable to cattle are used as cheap protein-rich material in all animal farms; the shell is generally simply recycled as fuel.

Polyphenols are highly valuable natural products [5]. They are able to protect cells from the damage caused by free radicals [6, 7]. They could also prevent atherosclerosis, cancer progression [8, 9], or pathogen development [10]. They are likely to be endowed with numerous other pharmacological properties [11]. Not surprisingly, argan oil poly- phenols are suspected to possess valuable therapeutic properties [12] and argan oil polyphenols have received special attention lately [13, 14]. Furthermore, a preparation composed of a crude flavonoid extract from argan leaves is currently marketed as preserving the skin against premature ageing [15]. Therefore, studying polyphenol content in argan oil by-product appears particularly appealing to further support, and possibly reinforce, the sustainable

development of the argan forest. Several phytochemical studies on argan tree parts or byproducts have already been carried out with particular attention to the polyphenols [16–22]. However, most of these studies were preliminary or only analytical and detailed quantification of polyphenol content in the different parts of argan material has never been reported. Furthermore, since those independent analyses were carried out on different material using different techniques, result comparison is almost impossible. Consequently, the true industrial potential of argan polyphenols remains still unknown. The purpose of this work was to identify and quantify on the same fruit batch the non-acidic phenolic content of the argan-nut shell, argan-fruit pulp, roasted and unroasted kernels, and derived press-cake, by HPLC technique coupled with ESI-MS (HPLC-ESI-MS). The antioxidant activity and radical scavenging capacity of these plant parts were evaluated with 2,2′- Azino-*bis*-(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt (ABTS) and diphenyl-1-picrylhydrazyl (DPPH·) assays, respectively.

2 Materials and methods

2.1 Chemicals

Standards of apigenin, (+)-catechin hydrate, (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate, fisetin, (-)-gallocatechin gallate, hesperidin, hyperoside, iso-quercitrin, luteolin, naringin, naringenin, myricetin, phloridzin, procyanidin B1, procyanidin B2, quercetin, quercitrin, and rutin were purchased from Sigma–Aldrich (Milan, Italy), as were DPPH, potassium persulfate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and formic acid. 2,2′-Azino-*bis*-(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt (ABTS), Folin–Ciocalteu (FC) reagent, and gallic acid were purchased from Fluka Chemicals (Milan, Italy). Acetone, methanol, *n*-hexane, and ethanol were of analytical or higher grade and purchased from Fluka Chemicals. Aqueous solutions were prepared using ultra-pure water produced with a Milli-Q System (Millipore, Milan, Italy).

2.2 Sample preparation

Argan fruit was collected in Ait Baha (Chtouka-Ait Baha, Morocco) in the summer of 2009. After harvest, fruit was dried and pulped. Resulting nuts were manually broken to separate argan shell from kernels. Approximately one half of the kernels was roasted for 30 min at 1108C using a mechanical roaster whose temperature was controlled using a Testo 945 sensor/thermometer (Testo, Casablanca, Morocco). Roasted and unroasted kernels were separately mechanically pressed using Komet DD 85 G presses (IBG Monforts Oekotec GmbH, Mö nchengladbach, Germany) affording the two types of press-cake.

2.3 Preparation of samples

Shell, pulp, roasted, and unroasted kernels and press-cake were separately frozen in liquid nitrogen, ground in a high-speed hammer mill (IKA A11 Basic, Germany) and individually defatted for 6 h with *n*-hexane in a Soxhlet apparatus. The resulting dough was dried in a rotary evaporator (Bü chi R-210 Flawil, Switzerland) under vacuum for 30 min at 458 °C to afford a powder that was extracted by adapting the method of Xu and Chang [23] with slight modifications. Briefly, 1 g of powder was placed in a 50 mL test tube containing 10 mL of a fresh mixture of acetone/water/formic acid (70:29.5:0.5 v/v/v). The suspension was shaken on an orbital shaker at RT for 3 h then centrifuged. The supernatant was collected in an amber vial and stored at +4°C. The residue was re-extracted for additional 12 h and extracts were combined. Acetone was evaporated under a nitrogen flux with stirring. Solutions were diluted to 5 mL with methanol/water/formic acid (50:49:1 v/v/v) and filtered (0.45 μm). Each sample was prepared in triplicate then used for the determination of total phenolic content (TPC), total antioxidant capacity, and the chromatographic analysis.

2.4 LC-MS analysis

A Thermo-Finnigan Spectra-System high-performance liquid chromatography (HPLC) system (Thermo-Finnigan, Waltham, USA), equipped with a P2000 binary gradient pump system, a SCM 1000 degasser, an AS 3000 automatic injector and a Finnigan MAT LCQ ion trap mass spectrometer with an ESI source was used. The separation was achieved on a Luna C18 column (150*2.0 mm^2, 5 µm; Phenomenex, Castel Maggiore, Italy). The mobile phase was composed of a mixture of solvent A (2% formic acid in water) and B (methanol). Flow rate was 0.2 mL min^{-1} and the injection volume 20 mL. Elution program was as follows: initial conditions solvent A 80%, concentration of solvent A was gradually decreased to 70% in 6 min. This ratio was kept for 14 min and concentration of solvent A was gradually decreased to 50% in 2 min then to 30% in 27 min. Concentration of solvent A was gradually decreased to 0% in 20 min and kept so for 5 min before to return to 80% in 2 min and held for 25 min. Negative electrospray mode was used for the ionization of molecules with spray voltage at 3.50 kV and capillary temperature at 2008 °C. The negative masses were monitored in the selected ion mode in 8 segments: *m/z* 289, 457, 577 from retention time (Rt) 0 to 11 min; *m/z* 441 from Rt 11 to 15 min; *m/z* 463, 579 and 609 from Rt 15 to 29 min; *m/z* 435 from Rt 29 to 31 min; *m/z* 285, 317, 447 from Rt 31 to 34 min; *m/z* 301 and 271 from Rt 34 to 38 min; *m/z* 285 from Rt 38 to 41 min; *m/z* 269 from Rt 41 to 44 min. Phenolic identification was obtained by comparing the Rt and mass spectrum (MS) with that of authentic standards. In addition, MS^2 experiments were carried out using helium as collision gas. Collision induced dissociation (CID) spectra were obtained with an isolation width of 1 *m/z* for parent mass and a normalized collision energy of 24% for procyanidin B1, procyanidin B2, (+)-catechin, (-)-epicatechin, 27% for (-)-epigallocatechin gallate, hesperidin, hyperoside, isoquercitrin and rutin, 29% for naringin, 32% for myricetin, naringenin, and quercetin. Quantification of each polyphenol was achieved using external calibration. Standards were individually dissolved in methanol/water/formic acid (50:49:1 v/v/v), diluted at 5000, 2000,

1000, 500, 100, 50, 20, and 10 *m*g/L, and used to provide the calibration curves. The limit of detection (LOD) was determined by using a signal-to-noise ratio of 3, while limit of quantification (LOQ) was calculated by using a signal-to-noise ratio of 10 (Table 1).

2.5 Spectrophotometric assays

2.5.1 Determination of total phenolic content (TPC)

The amount of total phenolics was assayed spectrophotometrically by means of the modified Folin–Ciocalteu (FC) method [24, 25]. Briefly, 2.5 mL of 10-fold diluted FC reagent, 2 mL of 7.5% aqueous sodium carbonate solution, and 0.5 mL of phenolic extract were mixed well. After 15 min of heating at 45 °C the absorbance was measured at 765 nm with a UV–Visible spectrophotometer (UV- 1700 PharmaSpec, Shimadzu, Milan, Italy) [26]. A mixture of solvent and reagents was used as a blank. The phenolic content was expressed as gallic acid equivalents (GAE) per g of sample, using a gallic acid standard curve (0–250 mg/L).

2.5.2 Determination of DPPH radical scavenging activity (RSA)

Hydrogen-donating ability of the crude extract and radical scavenging activity (RSA) of argan fruit parts were investigated using the DPPH· RSA [27, 28]. A volume of 3 mL of $6.1 * 10^{5}$ M DPPH· methanol solution was used. The reaction was started by the addition of 75 *m*L of sample extract. The purple color of the radical methanol solution changed gradually to yellow and the decrease in absorbance at 515 nm was recorded, at RT, until the absorbance was stable (1 h). All operations were done in the dark or dim light [29]. For control purpose, the absorbance of the DPPH· without samples was measured. The inhibition percentage (IP) of the DPPH· by the extracts was calculated according the formula $IP = [(A0min\ A60min)/A0min] * 100$ [30]

where A_{0min} is the absorbance of the control at t=0 min, and A_{60min} is the absorbance of the samples at 60 min. Ethanolic solutions of Trolox were used for calibration (0– 350 *m*M). The percentage of remaining DPPH· being proportional to the antioxidant concentration in the extracts, the DPPH· scavenging activity was expressed as *m*M of Trolox equivalent (TE) per mg of sample.

2.5.3 Determination of total antioxidant activity (TAA)

The total antioxidant activity (TAA) in crude extracts was determined according to the Trolox equivalent antioxidant capacity (TEAC) assay following the original analytical procedure described by Re et al. [31] with slight modifications. ABTS radical cation (ABTS·) was produced by reacting a 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration). The mixture was allowed to stand in the dark at RT for 12–16 h before use. The radical was stable in this form for more than 2 days when protected from light and stored at RT. For the study, the ABTS· stock solution was diluted with ethanol to an absorbance of 0.70 (0.02) at 734 nm, and equilibrated at 30°C. Sample solutions 30 *m*L (or standard) were mixed with ABTS· solution 3 mL. Absorbance readings were taken at 30°C exactly 6 min after initial mixing. Appropriate solvent blank was obtained by mixing absolute ethanol 30 *m*L with ABTS· solution 3 mL and monitored its absorbance at 6 min. All determinations were carried out in triplicate. The ABTS· scavenging effect (% Inhibition) was calculated by the equation: % Inhibition = [($A_{734blank}$ $A_{734sample}$)/ $A_{734blank}$] 100 where $A_{734blank}$ and $A_{734sample}$ are the absorbances of ABTS· solution at 734 nm before and after the samples addition. Calibration was performed, as described previously, with Trolox stock solutions. Results were expressed as *m*M TE per mg of sample.

2.5.4 Statistical analysis

Results were reported as mean SD (n=3). The one-way ANOVA was performed with SPSS software (version 12.0 for Windows, SPSS Inc., Chicago, Illinois). Duncan's test was applied to assess significant differences among the variables (p<0.05), while Pearson correlation test was used to show their correlations.

3 Results and discussion

Efforts were made to minimize losses of polyphenol antioxidant capacity during extraction. Therefore samples were freeze-dried and milling performed with a hammer mill as recommended [32]. Fruit processing, between harvest and polyphenol extraction, was performed using the exact methodology applied in argan oil production centers in order to get raw material whose quality really mimics that of argan oil by-products.

3.1 Identification and quantification of phenolic compounds in argan fruit parts

HPLC presents a lot of advantages for the separation and quantification of polyphenolics in fruits [33]. Some polyphenols have already been identified in argan parts [16–22]. Accordingly, those polyphenols were used as standards. Identification of the free phenolic compounds in argan fruit was carried out by HPLC-ESI-MS and achieved by comparing Rts and MS spectra with those of authentic standards. The MS spectrum showed the ions corresponding to the deprotonated molecule [MH], which provided the molecular weight of each compound. In addition, MS^2 was carried out to confirm the polyphenol identity. The MS^2 spectra afforded a fragmentation pattern specific for each molecule.
The LC–MS examination resulted in the detection of 12 components among which 11 were unambiguously identified. Identified compounds can be categorized into three groups: flavanols, flavonols, and dihydrochalcones. These components were found in various combinations and ratios (Table 2).

Six compounds were detected in the pulp. Isoquercitrin and hyperoside were predominant (28.4 and 21.1 mg/100 g, respectively) followed by rutin (9.8 mg/100 g) and quercitrin (0.2 mg/100 g). Epicatechin and procyanidin B2 were also detected but could not be quantified. In a previous qualitative study the presence of additional phenolic compounds had been detected in argan fruit pulp [17]. However, we were unable to reproduce this finding and neither hesperidin nor naringenin were found in the present work. This difference in composition between two independent studies suggests that argan fruit pulp phenolic composition is not constant, neither in qualitative nor quantitative terms, and could depend on genotypic factors. It also likely depends on several intrinsic and hard-to-control factors such as growing conditions, geographical origin, ripening process, ripening level, or storage conditions [34]. Such variations have already been observed for other fruit [35]. Finally, phenolic content discrepancy might also be the result from different extraction methods [36, 37].

Phenolics from argan fruit shell had never been studied before. The major phenolic compound isolated from the shell was (-)-epicatechin (0.6 mg/100 g). Others were iso-quercitrin (0.4 mg/100 g), rutin and phloridzin (both 0.2 mg/100 g), hyperoside, procyanidin B1 and B2 (both 0.10 mg/100 g), myricetin (0.05 mg/100 g), and finally quercitrin that was detected but could not be quantified.

Both roasted and unroasted kernels contained a large quantity of a yet unidentified compound (Mw=458.3; Rt: 8.9 min) as major component: 10.3 and 7.3 mg/100 g, respectively. MS fragmentation indicated that this compound was not (-)-epigallocatechin gallate despite their similar molecular weight. The polyphenolic nature of this compound remains dubious.

Unroasted kernels also contained (-)-epicatechin (0.6 mg/100 g), (+)-catechin (0.4 mg/100 g), hyperoside, isoquercitrin, epigallocatechin gallate, procyanidin B2 (both 0.2 mg/100 g), procyanidin B1 (0.1 mg/100 g), phloridzin (0.05 mg/100 g), rutin (0.02 mg/100 g), and minute and not quantifiable amount of quercitrin. Roasted kernels contained only five polyphenols: (+)-catechin (0.2 mg /100 g), (-)-

epicatechin (0.1 mg/100 g), and procyanidin B2 (0.1 mg/100 g). (-)-Epigallocatechin gallate and quercitrin were detected but could not be quantified. The low content in polyphenols of roasted kernels, compared to unroasted kernels, can likely be attributed to a temperature-assisted polyphenol degradation during the roasting step [28]. The unidentified compound observed in argan kernels was also found in the press-cake at a concentration of 6.4 and 5.1 mg/100 g for press-cake from unroasted and roasted kernels, respectively. (-)-Epicatechin was the only identified polyphenol in both type of press-cake. Similar levels were observed in both press-cakes (0.2 mg/100 g). Traces of procyanidin B1 and B2, myrcetin and quercitrin were detected, but not quantified, in both press-cakes. In addition, the press- cake produced from unroasted kernels also contained traces of (+)-catechin, (-)-epigallocatechin gallate and phloridzin. The phenolic fraction of the argan press-cake had been the sample, relatively to an equivalent reducing capacity of gallic acid. TPC of each part of argan fruit was measured by the FC assay and, as expected, argan fruit pulp also displayed the highest FC-TPC (75.8 mg GAE/g). Unroasted kernels and press-cake from roasted or unroasted kernels presented FC-TPC close to 8 mg GAE/g. Shell and roasted kernels FC-TPC was around 4 mg GAE/g (Table 2).

3.2 Radical scavenging activity

Argan fruit pulp that is the richest part argan fruit in poly- phenols displayed an antioxidant capacity of 0.17 *m*M Trolox Eq/mg (Table 3).

Radical scavenging activity of argan shell polyphenols was found to be higher than that of the kernels and press-cake (0.026 vs. 0.01 *m*M Trolox Eq/mg) but due to the presence of a unidentified compound, comparison with kernel data, as well as with other plant extracts, was difficult.

3.3 Total antioxidant activity

Total antioxidant activity, was determined by the TEAC assay that is based on the suppression of the absorbance of radical cations of ABTS· by antioxidants [38]. Polyphenols from argan fruit pulp displayed the highest measured TEAC (0.37 *m*M Trolox Eq/mg) followed by those of fruit shell and unroasted kernels (0.025 and 0.011 *m*M Trolox Eq/mg, respectively; Table 3).

3.4 Correlation between TAA and TPC

Total antioxidant activity of fruit is influenced by the degree of ripening [39, 40] and post-harvest phenomenon [41]. We found a good correlation between the TPC, determined by HPLC/MS, and the TAA of each parts of argan fruit (Table 4). Correlations were: R=0.994 between total phenolics by FC method and total phenolics by HPLC/MS, previously analytically studied by GC-MS after extraction and phenol silylation [16]. Such method had led to detect 16 polyphenols. Nine were detected during the present study. The difference observed between the two studies likely has the same origin as for the fruit pulp.

Total phenolic content (TPC) measured by HPLC indicated that argan fruit pulp was 30-fold richer in phenolics. Our results also showed that kernel roasting resulted in the loss of three-quarters of the total polyphenols (Table 2). We also decided to determine the TPC of our sample using the non-specific FC-TCP method because it is easy to implement and frequently used even though it rather provides information on the chemical reducing capacity of the sample, relatively to an equivalent reducing capacity of gallic acid. TPC of each part of argan fruit was measured by the FC assay and, as expected, argan fruit pulp also displayed the highest FC-TPC (75.8 mg GAE/g). Unroasted kernels and press-cake from roasted or unroasted kernels presented FC-TPC close to 8 mg GAE/g. Shell and roasted kernels FC- TPC was around 4 mg GAE/g (Table 2).

3.2 Radical scavenging activity

Argan fruit pulp that is the richest part argan fruit in polyphenols displayed an antioxidant capacity of 0.17 mM Trolox Eq/mg (Table 3).
Radical scavenging activity of argan shell polyphenols was found to be higher than that of the kernels and press-cake (0.026 vs. 0.01 mM Trolox Eq/mg) but due to the presence of a unidentified compound, comparison with kernel data, as well as with other plant extracts, was difficult.

3.3 Total antioxidant activity

Total antioxidant activity, was determined by the TEAC assay that is based on the suppression of the absorbance of radical cations of $ABTS^{\cdot}$ by antioxidants [38]. Polyphenols from argan fruit pulp displayed the highest measured TEAC (0.37 mM Trolox Eq/mg) followed by those of fruit shell and unroasted kernels (0.025 and 0.011 mM Trolox Eq/mg, respectively; Table 3).

3.4 Correlation between TAA and TPC

Total antioxidant activity of fruit is influenced by the degree of ripening [39, 40] and post-harvest phenomenon [41]. We found a good correlation between the TPC, determined by HPLC/MS, and the TAA of each parts of argan fruit (Table 4). Correlations were: R=0.994 between total phenolics by FC method and total phenolics by HPLC/MS, R=0.993 between $ABTS^{\cdot}$ assay and total phenolics by HPLC/MS, R=0.996 between $DPPH^{\cdot}$ assay and total phenolics by HPLC/MS. In addition, a significant linear relationship was found between the antioxidant capacity determined by $ABTS^{\cdot}$ and $DPPH^{\cdot}$ assays.

4 Conclusions

Taken together, our results evidenced that (i) compounds other than polyphenols are responsible for the antioxidant capacity of each studied argan

part and (ii) antioxidants formed during the roasting step (Maillard reaction products) have a strong antioxidant capacity since the FC-TCP of roasted kernels is less than one-half of that of unroasted kernels (4.8 mg GAE/g vs. 8.2 mg GAE/g) whereas its HPLC-calculated TPC is only one fifth that of unroasted kernels (0.4 mg/100 g vs. 1.97 mg/100 g) (Table 2).

Therefore, our results demonstrate the importance of the phenolic fraction on the antioxidant activity of argan fruit parts. Even though the composition of the phenolic fraction of argan fruit part may change over the years, crude argan fruit polyphenols deserve to be evaluated as nutraceutics, health supplements, or preservatives due to their antioxidant properties.

We thank the Slow Food Foundation for Biodiversity for its financial support and are grateful to the women of the Ait Baha co-operative and Association Ibn Al Baytar.

The authors have declared no conflict of interest.

References

[1] Charrouf, Z., Guillaume, D., Sustainable development in Northern Africa: The argan forest case. *Sustainability* 2009, *1*, 1012–1022.

[2] Charrouf, Z., Guillaume, D., Should the Amazigh diet (regular and moderate argan-oil consumption) have a beneficial impact on human health? *Crit. Rev. Food Sci. Nutr.* 2010, *50*, 473–477.

[3] El Monfalouti, H., Guillaume, D., Denhez, C., Charrouf, Z., Therapeutic potential of argan oil – A review. *J. Pharm. Pharmacol.* 2010, *62*, 1669–1675.

[4] Guillaume, D., Charrouf, Z., Argan oil and other argan products; use in dermocosmetology. *Eur. J. Lipid Sci. Technol.* 2011, *113*, 403–408.

[5] Balasundram, N., Sundram, K., Samman, S., Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.* 2006, *99*, 191–203.

[6] Zheng, R., Shi, Y., Jia, Z., Zhao, C., et al., Fast repair of DNA radicals. *Chem. Soc. Rev.* 2010, *39*, 2827–2834.

[7] Sies, H., Polyphenols and health: Update and perspectives. *Arch. Biochem. Biophys.* 2010, *501*, 2–5.

[8] Clement, Y., Can green tea do that? A literature review of the clinical evidence. *Prev. Med.* 2009, *49*, 83–87.

[9] Kar, P., Laight, D., Shaw, K. M., Cummings, M. H., Flavonoid-rich grapeseed extracts: A new approach in high cardiovascular risk patients? *Int. J. Clin. Pract.* 2006, *60*, 1484–1492.

[10] Scalbert, A., Morand, C., Manach, C., Rémésy, C., Absorption and metabolism of polyphenols in the gut and impact on health. *Biomed. Pharmacother.* 2002, *56*, 276–282.

[11] Núñez, M. J., Costoya, N., Natural antioxidants in health and diseases: A perspective. *E-J. Environ. Agric. Food Chem.* 2008, *7*, 3335–3342.

[12] Khallouki, F., Younos, C., Soulimani, R., Oster, T., et al., Consumption of argan oil (Morocco) with its unique profile of fatty acids, tocopherols, squalene, sterols and phenolic compounds should confer valuable cancer chemopreventive effects. *Eur. J. Cancer Prev.* 2003, *12*, 67–75.

[13] Marfil, R., Giménez, R., Martinez, O., Bouzas, P. R., et al., Determination of polyphenols, tocopherols, and antioxidant capacity in virgin argan oil (*Argania spinosa*, Skeels). *Eur. J. Lipid Sci. Technol.* 2011, *113*, 886–893.

[14] Cayuela, J. A., Rada, M., Pérez-Camino, D. C., Benaissa, M., et al., Characterization of artisanally and semiautomically extracted argan oils from Morocco. *Eur. J. Lipid Sci. Technol.* 2008, *110*, 1159–1166.

[15] Pauly, G., Henry, F., Danoux, L., Charrouf, Z., US patent 7,105,184 B2, 2006.

[16] Rojas, L. B., Quideau, S., Pardon, P., Charrouf, Z., Colorimetric evaluation of phenolic content and GC-MS characterization of phenolic composition of alimentary and cosmetic argan oil and press cake. *J. Food Agric. Chem.* 2005, *53*, 9122–9127.

[17] Charrouf, Z., Hilali, M., Jauregui, O., Soufiaoui, M., Guillaume, D., Separation and characterization of phenolic compounds in argan fruit pulp using liquid

chromatography– negative electrospray ionization tandem mass spectroscopy. *Food Chem.* 2007, *100*, 1398–1401.

[18] Chernane, H., Hafidi, A., El Hadrami, I., Ajana, H., Composition phénolique de la pulpe des fruits d'arganier (*Argania spinosa* L. skeels) et relation avec leurs caractéristiques morphologiques. *Agrochimica* 1999, *43*, 137–150.

[19] Chimi, H., Rahmani, M., Cillard, J., Cillard, P., Etude de la fraction phénolique des huiles d'olives vierges et d'argan du Maroc. *Actes Inst. Agr. Vét. (Maroc)* 1988, *8*, 17–21.

[20] Tahrouch, S., Andary, C., Rapior, S., Mondolot, L., et al., Polyphenol investigation of *Argania spinosa* (Sapotaceae) endemic tree from Morocco. *Acta Bot. Gall.* 2000, *147*, 225–232.

[21] El Kabouss, A., Charrouf, Z., Oumzil, H., Faid, M., et al., Caractérisation des flavonoÿdes des feuilles de l'arganier (*Argania spinosa*) et étude de leur activité antimicrobienne. *Actes Inst. Agr. Vét. (Maroc)* 2001, *21*, 157–162.

[22] Charrouf, Z., Guillaume, D., Phenols and polyphenols from *Argania spinosa*. *Am. J. Food Technol.* 2007, *2*, 679–683.

[23] Xu, B. J., Chang, S. K., A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J. Food Sci.* 2007, *72*, 159–166.

[24] Singleton, V. L., Rossi, J. A., Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 1965, *16*, 144–158.

[25] Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M., Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 1999, *299*, 152–178.

[26] Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., Nú ñ ez, M. J., Effect of solvent, temperature and solvent-to-solid ratio on the total phenolic content and antiradicalary activity of extracts from different components of grape pomace. *J. Agric. Food Chem.* 2005, *53*, 2111–2117.

[27] Von Gadow, A., Joubert, E., Hansmann, C. F., Comparison of the antioxidant activity of aspalathin with that of other plant phenols of Rooibosed tea (*Aspalathus linearis*), *a*-tocopherol, BHT and BHA. *J. Agric. Food Chem.* 1997, *45*, 632–638.

[28] Schinella, S. M., Cienfuegos-Jovellanos, E., Pasamar, M. A., Muguerza, B., et al., Antioxidant properties of polyphenol- rich cocoa products industrially processed. *Food Res. Int.* 2010, *43*, 1614–1623.

[29] Sharma, O. P., Bhat, T. K., DPPH antioxidant assay revisited. *Food Chem.* 2009, *113*, 1202–1205.

[30] Yen, G. C., Duh, P. D., Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. *J. Agric. Food Chem.* 1994, *42*, 629–632.

[31] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., et al., Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free Rad. Biol. Med.* 1999, *26*, 1231–1237.

[32] Pérez-Jiménez, J., Arranz, S., Tabernero, M., Diaz-Rubio, M. E., et al., Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Res. Int.* 2008, *41*, 274–285.

[33] Ignat, I., Volf, I., Popa, V. I., A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chem.* 2011, *126*, 1821–1835.

[34] Hilali, M., Charrouf, Z., Soulhi, A. E. A., Hachimi, L., Guillaume, D., Influence of origin and extraction method on argan oil physicochemical characteristics and compo- sition. *J. Agric. Food Chem.* 2005, *53*, 2081–2087.

[35] Naczk, N., Shahidi, F., Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *J. Pharm. Biomed. Anal.* 2006, *41*, 1523–1542.

[36] Franco, D., Sineiro, J., Rubilar, M., Sanchez, M., et al., Polyphenols from plant materials: Extraction and antioxidant power. *E-J. Environ. Agric. Food Chem.* 2008, *7*, 3210–3216.

[37] Prior, R. L., Wu, X., Schaich, K., Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* 2005, *53*, 4290–4302.

[38] Rice-Evans, C. A., Miller, N. J., Paganga, G., Structure- antioxidant activity relatioships of flavonoids and phenolic acids. *Free Rad. Biol. Med.* 1996, *20*, 933–956.

[39] Olsson, M. E., Ekvall, J., Gustavsson, K. E., Nilsson, J., et al., Antioxidants, low-molecular weight carbohydrates, and total antioxidant capacity in strawberries

(Fragaria x ananassa): Effects of cultivar, ripening, and storage. *J. Agric. Food Chem.* 2004, *52*, 2490–2498.

[40] Prakash, D., Upadhyay, G., Singh, B. N., Singh, H. B., Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (*Glycine max*). *Food Chem.* 2007, *104*, 783–790.

[41] Srivastava, A., Akoh, C. C., Yi, W., Fischer, J., Krewer, G., Effect of storage conditions on the biological activity of phenolic compounds of blueberry extract packed in glass bottles. *J. Agric. Food Chem.* 2007, *55*, 2705–2713.

Correspondence: Prof. Dominique Guillaume, UFR Mé decine- Pharmacie, 51 Rue Cognacq Jay, 51100 Reims, France

E-mail: dominique.guillaume@univ-reims.fr

Fax: +33 326918029

Abbreviations: ABTS, 2,2'-azino-*bis*-(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FC, Folin– Ciocalteu; GAE, gallic acid equivalents; RSA, radical scavenging activity; Rt, retention time; TAA, total antioxidant activity; TEAC, Trolox equivalent antioxidant capacity; TPC, total phenolic content; Trolox, 6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid

Table 1. Regression data, LOD, LOQ for phenolic compounds in argan parts

	Calibration curve	R^2	Linear range (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Procyanidin B1	Y=6 000 000x + 159 370	0.9836	50–2000	10	50
(+)-Catechin	Y=792 361x + 72 176	0.9992	50–2000	10	50
Procyanidin B2	Y=5 000 000x + 284 603	0.9931	50–2000	50	100
(-)-Epigallocatechin Gallate	Y=1 000 000x + 89 051	0.9980	50–2000	20	200
(-)-Epicatechin	Y=804 302x + 145 920	0.9979	50–2000	10	80
Isoquercitrin	Y=30 000 000x + 302 815	0.9996	50–5000	10	20
Hyperoside	Y=30 000 000x + 302 815	0.9996	50–5000	10	20
Rutin	Y=60 000 000x + 1 000 000	0.9998	50–5000	10	20
Phloridzin	Y=10 000 000x + 33 993	0.9979	30–1000	6	10
Myricetin	Y=50 000 000x + 8 000 000	0.9950	50–5000	10	30
Quercitrin	Y=60 000 000x + 10 000 000	0.9812	30–5000	10	30

Table 2. Total and individual phenolic compounds detected in the different parts of argan fruit by HPLC

Polyphenols	Shell	Pulp	Unroasted kernels	Roasted kernels	Press-cake (unroasted kernels)	Press-cake (roasted kernels)
Procyanidin B1	0.1±0.05^{a}	ND	0.1±0.05^{a}	ND	NQ	NQ
(+)-Catechin	ND	ND	0.4±0.05^{f}	0.2±0.05^{a}	NQ	ND
Procyanidin B2	0.1± 0.05^{a}	NQ	0.2±0.05^{a}	0.1±0.05^{a}	NQ	NQ
(-)-Epigallocatechin Gallate	ND	ND	0.2±0.1^{a}	NQ	NQ	ND
Unknown (Mw ¼ 457.3)	ND	ND	7.3±0.8^{d}	10.3±4.3^{e}	6.4±0.8^{c}	5.1±0.4^{c}
(-)-Epicatechin	0.6±0.1^{f}	NQ	0.6±0.1^{f}	0.1±0.05^{a}	0.2±0.1^{a}	0.2±0.1^{a}
Isoquercitrin	0.4±0.1^{f}	28.4±2.2^{g}	0.2±0.1^{a}	ND	ND	ND
Hyperoside	0.1±0.05^{a}	21.1±3.3^{f}	0.2±0.05^{a}	ND	ND	ND
Rutin	0.2±0.1^{a}	9.8±0.7^{e}	0.02±0.01^{b}	ND	ND	ND
Phloridzin	0.2±0.1^{a}	ND	0.05±0.01^{b}	ND	NQ	ND
Myricetin	0.05±0.03^{b}	ND	ND	ND	NQ	NQ
Quercitrin	NQ	0.2±0.1^{a}	NQ	NQ	NQ	NQ
TPC (HPLC method)	1.75±0.6^{h}	59.5±6.3^{i}	1.97±0.6^{h}	0.4±0.15^{j}	0.2±0.1^{j}	0.2±0.1^{j}
TPC (FC method)	4.4±0.5^{k}	75.8±0.8^{m}	8.2±0.7^{l}	4.8±0.2^{k}	7.9±0.6^{l}	7.3±0.6^{l}

Concentration is expressed in mg/100 g or mg GAE/g for measurements by the FC method.
Data are expressed as mean SD (n=3); ND: not detected; NQ: not quantified; Values followed by different letters are significantly different (p<0.05).

Table 3 . Radical scavenging and antioxidant acidity of the crude polyphenols extracted from different parts of argan fruit

Argan fruit part	Radical scavenging activity (µM TRolox Eq/mg)	TE antioxidant activity (µM Trolox Eq/mg)
Pulp	0.17±0.005^{b}	0.37±0.07^{b}
Shell	0.026±0.003^{a}	0.025±0.005^{a}
Unroasted kernel	0.01±0.002^{a}	0.011±0.002^{a}
Roasted kernels	0.009±0.001^{a}	0.006±0.001^{a}
Press-cake from unroasted kernels	0.01±0.001^{a}	0.009±0.001^{a}
Press-cake from roasted kernels	0.008±0.001^{a}	0.010±0.001^{a}

Data are expressed as mean ±SD (n=3). Means ±SD in a column with different letters are statistically different (p<0.001)

Table 4. Pearson correlation coefficient (*R*) between antioxidant capacity assays and TPC

R	TPC	RSA	TEAC
RSA	0.988		
TEAC	0.983	0.988	
TPC by HPLC	0.994	0.993	0.996